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Effects of esmolol on lung injury induced by lower extremity Ischemia-reperfusion

The effects of esmolol on lung injury

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Abstract
Aim: After hind limb ischemia-reperfusion (I/R), impairments in remote organs are frequent. Lung tissue is the organ most affected by the remote organ damage. The lung damage increases ventilatory support, the need for inotropic agents and mortality. Many drugs and methods have been used in attempts to prevent or reduce this damage. The aim of this study is to investigate the protective effects of esmolol infusion on lung tissue prior to I/R created in the lower extremity.
Material and Methods: The study was performed between 11 and 14 April 2018 in Gazi University Experimental Animal Research Center, Ankara, Turkey. After obtaining ethics committee approval, 24 rats were randomly divided into 4 groups: Control (Group C), Esmolol (Group E), Ischemia-reperfusion (Group I/R), and I/R-Esmolol (Group I/RE). Esmolol (200 µg/kg/min intravenous) was applied 30 minutes before the procedure. The biochemical and histopathological parameters of lung tissue samples were compared.
Results: Neutrophil infiltration/aggregation, alveolar wall thickness, and total lung injury scores were significantly higher in the I/R group than in the C and E groups. In addition, neutrophil infiltration/aggregation, alveolar wall thickness, and total lung injury scores in the I/R group were statistically higher than in the I/R-E group (p=0.030, p=0.010, p=0.001, respectively). Malondialdehyde levels, catalase (CAT) and paraoxonase (PON) enzyme activities in the I/R group were significantly higher than in the C, E, and I/R-E groups. Glutathione S- transferase (GSH) enzyme activity was similar in all groups.
Discussion: It was found that esmolol infusion at 200 µg/kg/min intravenously-reduced oxidative stress when administered 30 minutes before ischemia in rats and partially corrected the damage caused by I/R in lung histopathology.

Keywords
Reperfusion Damage, Ischemia-Reperfusion, Lung Injuries, Beta-Antagonists

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Introduction

Following an ischemic period, oxygen supply is not sufficient to meet the metabolic needs of cells in the ischemic area, and anaerobic metabolism is therefore activated. When blood flow is regenerated after ischemic period, tissue damage increases with the reestablishment of oxygen supply. Additionally, an inflammatory cascade that affects the distant organs begins. Blood, kidney, and lung tumor necrosis alpha (TNF-α), interleukin (IL) IL-1 and IL-6, as well as cytokines, all play a role in inflammatory cascade and cause local and distant organ damage via oxygen free radicals and leukocytes [1]. The lungs are the most affected remote organs by ischemic damage. As a result, noncardiogenic pulmonary edema is observed and alveolar membrane permeability increases. Partial atelectasis with collapsed-pinched alveoli and thicker -felted alveolar walls lead to decreased PaO2/ FIO2 ratio and impaired oxygenation, which increases patient mortality [2,3] .

Recently, various agents that reduce lower extremity-mediated lung injury have been studied [4-8] . Esmolol, however, which is a selective beta (β)-1 adrenoreceptor blocker, has not been studied. Esmolol has been proven to reduce ischemia-reperfusion (I/R) injury in cardiac surgery settings and spinal cord I/R injuries [9,10] . This study aimed to evaluate the protective effects of esmolol on lower extremity mediated lung I/R injury. Our hypothesis was that esmolol might reduce the lung damage caused by I/R injury. Lung injury was evaluated by catalase (CAT), glutathione S-transferase (GST) and paraoxonase (PON) enzyme activities, and malondialdehyde (MDA) levels. Additionally, histopathological evaluation of the tissue samples was performed.

Material and Methods

Animals:

The study was performed between April 11 and April 14, 2018; in Gazi University Experimental Animal Research Center, Ankara, Turkey. The procedures were performed in accordance with the proper use and care of laboratory animals, approved by the ethics committee of GUDAM (G.U.E. T-17.082-07.11.2017). Experiments were performed on 24 male Wistar rats weighing 250-330 g. The animals were maintained under standard conditions such as stable room temperature (24 ± 3 ºC) and a 12-hour light-dark cycle and were allowed access to rat pellets and water.

Experimental Model:

Rats were anesthetized with intraperitoneal (ip) ketamine (100 mg/kg) in all groups. Maintenance of anesthesia was done with ip ketamine 20 mg/kg during the procedures. The tail vein was cannulated for hydration, if necessary, with a 24 Gauge cannula. Esmolol (200 µg /kg) was administered 30 minutes before the operation and esmolol infusion duration was 15 minutes without a bolus dose. A middle abdominal incision was done and the intestines were removed to maintain a clear vision of the infrarenal aorta. An atraumatic microvascular bulldog clamp was placed on the infrarenal abdominal aorta, in the I/R and I/R-E groups. The surgery took 10±3minutes. After 120 minutes, the clamp was removed and reperfusion was performed for 120 minutes [11] . Sodium heparin (500 IU/kg) was administered through the peripheral vein in the tail for the maintenance of reperfusion after occlusion [12] . All rats were euthanized by drawing blood intracardially and tissue samples were obtained.

Experimental Protocol:

Group C: Control group (n=6)

Group E: Esmolol group (n=6)

Group IR: Ischemia-reperfusion group (n=6)

Group IR-E: Ischemia-reperfusion- esmolol group (n=6)

Group C: In this group, anesthesia was induced with ketamine followed by iv cannulation. Abdominal incision without intestinal removal was applied, and no further application was performed. Blood samples were obtained 120 minutes after the commencement of reperfusion by drawing blood intracardially, and tissue samples were obtained.

Group E: Following anesthesia, the rats' tail veins were cannulated. After this, esmolol (200 µg/kg) was administered 30 minutes before the operation and esmolol infusion duration was 15 minutes. Thirty minutes after the infusion, the I/R model was performed. Blood samples were obtained after 120 minutes of reperfusion by drawing blood intracardially, and tissue samples were obtained.

Group I/R: Rats were given 100 mg/kg of ketamine, and their tail veins were cannulated. Following 15 minutes without any application, the I/R model was performed. Blood samples were obtained at the end of the I/R model by drawing blood intracardially. Subsequently, tissue samples were obtained.

Group I/R-E: Rats were given 100 mg/kg of ketamine, and their tail veins were cannulated. After the cannulation, esmolol (200 µg/kg) was administered 30 minutes before the operation and esmolol infusion duration was 15 minutes. Then I/R model was performed. Blood samples were obtained at the end of the I/R model by drawing blood intracardially. Finally, tissue samples were obtained.

After this reperfusion time, biochemical and histopathological evaluations of lung tissue specimens were performed. The right lung was used for histopathological evaluation and the left was used for biochemical evaluation. Histopathological assessment was performed in the Kırıkkale University Medical Faculty Histology and Embryology Department. Paraffin blocks were formed by the specimens after a routine fixation process. Tissue sections were mounted on slides for staining with hematoxylin and eosin (H&E).

Histopathological Assessment of the Lung

A researcher uninvolved in the study examined lung samples histopathologically using light microscopy. Worst and best areas were evaluated using microscopy in H&E stained sections. Neutrophil infiltration and alveolar thickness were measured in each specimen to expose the degree of lung injury. Each parameter was scored as none (0 points), quite little (1 point), medium (2 points), or severe (3 points). Two scores were added and noted as the final lung injury score [13].

Biochemistry

The biochemical analysis was done in the Gazi University Medical Faculty Biochemistry Department. The lung tissue was first washed with cold deionized water to discard blood contamination and then homogenized in a homogenizer.

Measurement of MDA levels, thiobarbituric acid (TBA) reactive substances assay was performed using the method described in the study by Van Ye et al [14]. The CAT activity is based

on the measurement of absorbance decrease due to H2O2 consumption at 240 nm by Aebi H method [15]. GST enzyme activity was measured using the method described by Habig et al. [16] . PON-1 activity was measured at the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25 °C by Brites FD. Method [17] .

The sample protein amount was determined by the Lowry O method, and BSA was used as the standard protein [18].

Statistical analysis

All data were expressed as mean and standard deviation (SD). Comparisons between multiple groups were performed using the Kruskal–Wallis test. Differences were identified using the Bonferroni corrected Mann–Whitney U test. SPSS 12. 0 for Windows (SPSS Inc., Chicago, IL, USA) was used to complete all the analyses, and $P < 0.05$ was considered statistically significant.

Results

Histopathologically, neutrophil infiltration/aggregation, alveolar wall thickness and total lung injury score were significantly higher in the I/R group when compared to the C and E groups. In addition, neutrophil infiltration/aggregation, alveolar wall thickness, and total lung injury scores in the I/R group were statistically higher than in the I/R-E group (p=0.030, p=0.010, p=0.001, respectively) (Table 1, Figures 1-3).

CAT and PON enzyme activities and MDA levels were significantly higher in the I/R group compared to the C, E and I/R-E groups. GST enzyme activity was similar between the groups (Table 2).

Discussion

Our experimental study on hind-limb I/R injury-induced lung injury in rats showed that esmolol was effective in preventing the I/R induced lung injury. Esmolol (200 µg/kg) administration 30 minutes before the operation for 15 minutes of infusion reduced the neutrophil infiltration/ aggregation and alveolar wall thickness in lung tissue induced by hind-limb I/R.

Dupeng et al. have studied the protective effects of esmolol and its possible mechanism of protection on lungs in septic rats. They established the sepsis model with cecal ligation and infused esmolol for six hours in a dose of 15mg/kg per hour continuously. They measured the levels of catecholamine (CA) in plasma and levels of NF-κB and TLR4 in lung tissue. Additionally, they evaluated the protein content of alveolar lavage fluid, wet/dry weight ratio (W/D) of lung tissue, lung coefficient and lung water content, finding that protein content of alveolar lavage fluid, W/D in lung tissue, lung coefficient, and lung water content were significantly lower in the esmolol groups. They conclude that esmolol can promote the secretion of catecholamine, inhibit the release of inflammatory cytokines, reduce lung permeability, and reduce pulmonary edema. The mechanism may be that esmolol improves the responsiveness of the β-adrenergic receptor to catecholamine, and regulates the level of inflammatory cytokines by inhibiting TLR4-NF-κB-TNF-α signaling pathways, thus exerting a protective effect on the lungs [19]. We observed similar results in our study on lung tissue histopathologically.

Esmolol has been previously studied in lung resection

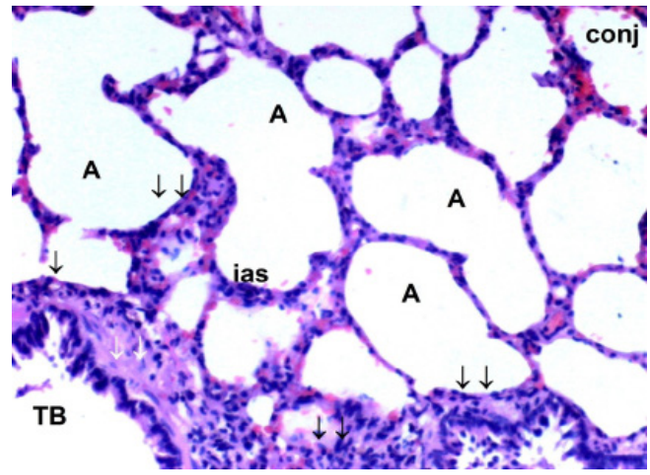

Figure 1. Normal lung tissue, control group, HeX100. A: alveoli, TB: terminal bronchiole, SA: saccus alveolaris, IAS: interalveolar septum, ↓↓: Septum thickening, ↓: Pulmonary vessel thickening, conj: Capillary congestion

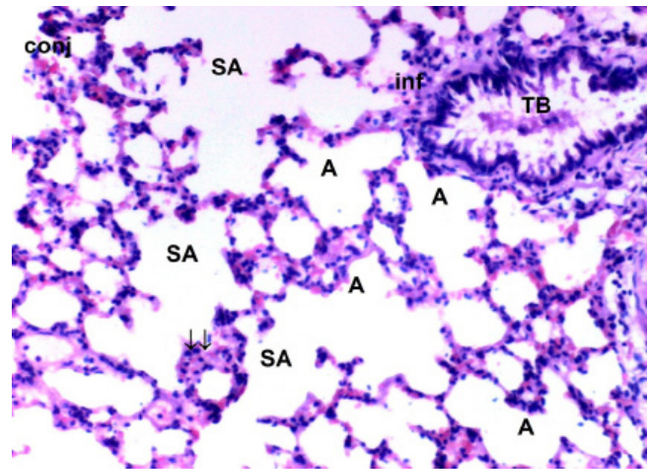

Figure 2. Severe neutrophil infiltration and increased alveolar wall thickness, I/R group,Hex100. A: alveoli, TB: terminal bronchiole, ↓↓: Septum thickening, Conj: Capillary congestion, Inf: Inflammation, neutrophil infiltration (white arrow)

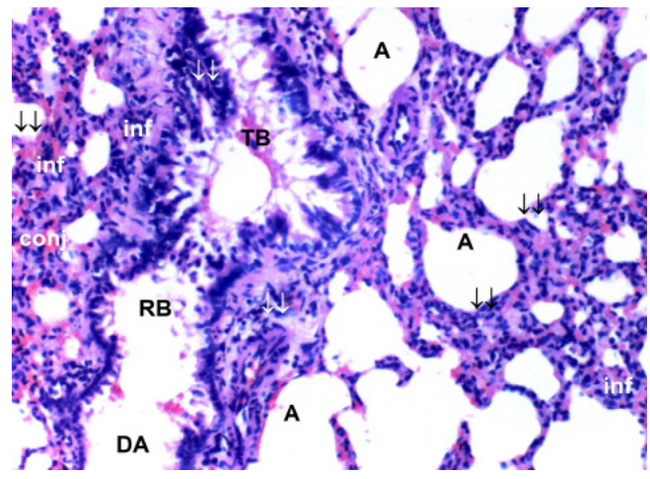

Figure 3. Mild neutrophil infiltration and increased alveolar wall thickness, I/R-E group, Hex100. A: alveoli, TB: terminal bronchiole, RB: respiratory bronchiole, DA Ductus alveolaris (alveolar canal), ↓↓: Septum thickening, Conj: Capillary congestion, Inf: Inflammation, neutrophil infiltration (white arrow)

Table 1. Histopathological findings of rat lung tissue [Mean ± SD]

	Group C (n=6)	Group E (n=6)	Group I/R (n=6)	Grup I/R-E (n=6)	P**
Neutrophil infiltration/ aggregation	0.33±0.21*	0.50±0.22*	2.33±0.33	1.50±0.22*,+	<0.0001
Alveolar wall thickness	0.33±0.21*	0.67±0.33*	2.33±0.21	1.33±0.21*,+	<0.0001
Total score	0.67±0.21*	1.17±0.40*	4.67±0.49	2.83±0.17*,+	<0.000

p **: Significance level with Kruskal Wallis test; p <0.05. * p <0.05: When compared to Group I/R, + p <0.05:When compared to Group C

Table 2. Lung tissue oxidant status parameters of rats [Mean ± SD]

	Group C (n=6)	Group E (n=6)	Group I/R (n=6)	Group I/R-E (n=6)	P**
MDA (nmol/mL)	3.10±1.04*	4.16±1.01*	6.77±1.61	4.45±1.20*	0.001
CAT (IU/mg.pro)	303.60±66.17*	320.40±59.02*	467.29±148.35	302.17±114.76*	0.38
GST (IU/mg.pro)	1.54±0.69	1.53±0.95	2.27±0.71	1.58±0.49	0.216
PON (IU/mg.pro)	7.72±0.98*	7.32±2.33*	9.90±1.67	6.28±1.55*	0.008

p **: Significance level with the Kruskal-Wallis test p <0.05. * p <0.05: When compared to Group I/R

surgery (LRS) in porcines. The researchers analyzed the effect of a continuous perioperative iv infusion of esmolol on postoperative pulmonary edema in an experimental model of LRS, requiring periods of one-lung ventilation (OLV). They studied lung biopsies and plasma samples to analyze the levels and expression of inflammatory mediators, and the animals also received a bronchoalveolar lavage. The authors concluded that the esmolol treated group had less lung edema and lower expression of the proinflammatory biomarkers TNF and IL-1 compared to the control group for both lung lobes. They suggest that esmolol reduces lung edema and inflammatory responses in intraoperative and postoperative periods in animals that underwent LRS with OLV [20]. In our study we did not analyze the same biomarkers, but we observed decreased MDA, CAT, and PON levels in rats. Likewise, we observed less lung injury in histopathologic evaluation.

Esmolol has been studied for its effects in early sepsis for multiple organ functions and has been shown to reduce apoptosis and inflammation and decrease serum IL-6, HMGB-1 and TNF- α levels. Moreover, it protected key organs [21]. The protective effect of esmolol on lung tissue has been shown in a model of severe acute pancreatitis, the esmolol treatment reduced bronchoalveolar lavage fluid protein and proinflammatory cytokines TNF-α and IL-6 levels, decreased pancreatic/lung MPO activities. Furthermore, they showed clinical improvement in rats, concluding that esmolol has protective effects both on the lung and pancreas [22].

Increased levels of superoxide radicals (SOR) have an important role in the I/R injury caused by infrarenal occlusion of the aorta. These SORs are aligned as superoxide (O2-), hydrogen peroxide (H2O2) and hydroxyl (OH-) ions. The superoxide is converted to H2O2 with the catalyst of superoxide dismutase and then the H2O2 is converted to H2O and CO2 with the catalysts of glutathione peroxidase and CAT and H2O2 is therefore inactivated. Thus CAT is an intracellular enzyme, which has a key role in deactivating SORs. Also, MDA is a sensitive marker and a final product of cell membrane lipid peroxidation. Gulmen et al. concluded in their study with lower extremity I/R model that MDA and CAT levels were increased in I/R injury groups [23]. Kapan et al. also observed increased levels of CAT and MDA in their lung injury model induced by their infrarenal abdominal aorta I/R injury model. In their model, they applied 30 minutes of ischemia and 60 minutes of reperfusions and observed increased MDA and CAT levels [24]. In our study, in I/R injury groups, we observed increased CAT and MDA levels, which are defined as important and delicate markers of injury, also in esmolol treated groups we observed decreased levels of these indicators. In our study, the GST levels were higher in I/R injury groups and lower in esmolol treated groups but these levels were not significant.

Current knowledge about the dosage varies in research. Umehara et al. infused 200 μg/kg/min, and Zhang et al. infused 15 mg/kg per hour. We used the same dosage as Umehara et al. and obtained beneficial effects [9,22]. Umehara et al. infused esmolol 30 minutes before the I/R model, as we did, but they continued infusion for the subsequent 24 hours [9]. We prefer not to infuse for 24 hours to observe the effects of infusion when done only prior to surgery and observed beneficial effects. In this study, esmolol reduced lung injury significantly in the I/R-E groups when compared to I/R group. Zhang et al. induced severe acute pancreatitis in rats and they evaluated the effects of esmolol in these rats on lung tissue. In these rats they observed increased diffuse edema formation, interstitial infiltration by neutrophils and reduced alveolar spaces in the SAP group. Histology scores, based on the number of areas with congestion, edema, inflammatory cells, and hemorrhaging were lower in esmolol-treated animals than in SAP animals [22].

There are some limitations in the research. We only studied GST, CAT, PON enzyme activities and MDA levels because of technical incapability, but TNF-α, IL 1, IL 6 could be studied as well to support our findings. Also, different doses of esmolol have been defined to be effective in lung injury, but we infused 200 μg/kg/min dose and found it to be effective, other doses can be studied as well. The lack of data about hemodynamical changes in rats regarding esmolol infusion is another limitation of the study.

Conclusion

In conclusion, in our experimental study, hind-limb I/R injury-induced lung injury in rats showed that esmolol was effective in preventing the IR-induced lung injury. Esmolol (200 μg/kg)

administration 30 minutes before the operation for 15 minutes reduced the neutrophil infiltration/aggregation and alveolar wall thickness in lung tissue induced by hind-limb I/R. Increased levels of GST, CAT, MDA and PON in lung tissues supported by I/R are mediated via oxidative reactions and decreased levels of CAT, MDA and PON in esmolol treated groups supported our histopathological findings. Such lung injury treatments can be used to enhance lung functions following hind-limb I/R.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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